Annual Progress Report

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DIETARY FACTORS AFFECTING EXOGENOUS AND ENDOGENOUS SOURCES OF FAT AND CARBOHYDRATE FOR ENERGY PRODUCTION AND SYNTHESIS

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In planning for man's extended periods of time in space flight, the special dietary requirements imposed by inactivity, lack of gravitational force, and limited storage space must be considered.

Fat is the most concentrated form of energy which can be supplied to living organisms. This form of energy may be provided exogenously by food or endogenously by fat synthesis from otherwise unused carbohydrate, protein, or fat. Questions concerning the relative efficiencies of calorie sources--exogenous or endogenous--during extended periods of caloric expenditure with limited accessibility of food seem relevant--e.g., What are the relative efficiencies of fat utilization for energy when it is supplied exogenously in food or from body stores within the animal? Are the caloric requirements best met by eating fat during the time of need or from body fat stores? Are some fatty acids more readily used than others for energy?

The purpose of the present investigation undertaken during the period under review, was to study the effects of diet composition on a) total fatty acid content of liver, adipose tissue and carcass, b) changes in fatty acid composition of liver, adipose tissue, and carcass, c) activities of enzymes related to fat and carbohydrate metabolism, and d) rate of deposition or utilization of body fat in rats given purified diets at certain levels of caloric intake.

EXPERIMENT I

Procedure:

Animals. Weanling 21-day-old female rats of the Long-Evans strain were housed individually and fed purified diets containing 0%, 11%, or 45% of the calories as fat for 11 weeks. The composition of the diet is shown in Table 1. During this 11 weeks the rats were weighed and given 4 drops of safflower oil twice weekly. The ad libitum food intake was also measured twice weekly.

At 11 weeks post-weaning the three groups were divided as follows:

Initial Diet for 11 weeks	No. of Rats	Number of rats on final diets (5.5 weeks)					
		25 cal/100 gm. body wt/day			19 cal/100 gm. body wt/day		
		0% Fat	11% Fat	45% Fat	0% Fat	11% Fat	45% Fat
0% Fat	9	1	0	2	3	0	3
11% Fat	14	5	0	5	0	4	0
45% Fat	20	3	5	5	2	0	5

Body weights were recorded twice weekly, the safflower oil was continued and the diets were rationed so that the rats received 25 or 19 cal/100 gm. body weight/day.

At the end of the final diet period (5.5 weeks) the food cups were removed at 5 PM the day before sacrifice. Sacrifice was by guillotine in the morning. Blood was drained from the neck and internally after cutting the hepatic artery, liver was removed and weighed, all of the visible fat in the abdominal cavity was removed and weighed as abdominal fat or mesenteric fat, and the gastrointestinal tract was discarded. The carcass (minus liver, abdominal fat and gastrointestinal tract) was weighed.

Chemical methods. The liver and fat samples were dried in vacuo at room temperature to constant weight (about 70 hours) for determination of solids. The total fatty acids were then extracted from the dried samples with hot ethanol-ether (3:1), saponified, methylated and their fatty acid composition analyzed by gas-liquid chromatography (1).

Carcass was digested in 400 ml of 25% alcoholic KOH on the steambath for about 4 hours. The volume was then made up to 1 liter with 45% ethanol and aliquots were taken for fatty acid extraction. Fatty acid composition was determined as given above (1).

Results

Table 2 shows the effects of varying the caloric intake on body weight. Utilization of calories as evaluated by changes in body weight appeared to be the same whether 0%, 11%, or 45% of the calories were supplied by fat. Groups receiving 25 cal/100 gm. body weight gained about 0.5 gm per day, while those receiving 19 cal/100 gm body weight lost about 0.8 gm per day.

Fatty acid composition of liver (Table 3) was very different from that of abdominal fat (Table 4) or carcass (Table 5) as shown in Table 6. This is to be expected since phospholipid is the chief lipid component of liver whereas in adipose tissue it is mainly triglyceride. In liver, C12:0 and C14:0 fatty acids were higher and the C18:1 fatty acid lower when the 45% fat diet was fed rather than the 0% fat diet. The changes, however, were small compared with the differences in the carcass or abdominal fat. Rats fed 11% fat from weaning or those whose diet was changed to the 0% or 11% diet from the 45% fat diet had C12:0 and C14:0 fatty acid values between those for 0% and 45% fat diets.

The solids and fatty acid content of the liver, abdominal fat and carcass from the rats on the different diet regimens are shown in Tables 3, 4, 5. These data are summarized according to diet groups in Table 6 for liver and in Table 7 for abdominal fat and carcass. The % of solids in liver was 30-32% and the % of total fatty acids (TFA) in liver (dry weight) was 12-14%. There appeared to be no difference in % solids with respect to diet composition or level of caloric intake, but the % TFA appeared to be higher in groups fed the 11% fat diet the entire time or changed from 45% to 11%.

There may have been a slightly lower % solids in abdominal fat in rats fed 3/4 as many calories as the full ration as indicated in table 4. The values averaged 86% compared with 93% for the full-calorie feeding. Also, the TFA level was about 92% of solids for the full-ration rats compared with 89% for those fed 3/4 rations.

The rats fed 3/4 calorie diets may have had a slightly lower % of TFA in the carcass (Table 5). The value was about 8% versus 11% of wet carcass weight in the full-fed rats.

From tables 6 and 7 it may be seen that the rats fed 3/4 as many calories as the full ration rats for 5-6 weeks after ad libitum feeding for 3 months had the same fatty acid composition of carcass, abdominal fat or liver as did those given full rations during the final 5.5 weeks.

Fatty acid composition of carcass and abdominal fat were alike for a given dietary regimen except that carcass had a higher percentage of linoleic and arachidonic acid (Table 7). Fatty acid composition of carcass and abdominal fat from rats fed 45% of the calories as hydrogenated coconut oil contained the highest proportions of C12:0, C14:0, and C14:1 fatty acids and the lowest proportions of C16:1 and C18:1 fatty acids. The fatty acid

values were similar whether the rats had consumed the 45% fat ration since weaning or had been transferred from 0% or 11% fat diets to the 45% fat diet. The fatty acids of abdominal and carcass fat of rats fed diets providing 11% of the calories had values for C12:0, C14:0, and C18:1 fatty acids between those for rats fed the 0% and 45% fat diets. Rats fed 45% fat diets at first and then changed to 0% or 11% fat diets for the last 5.5 weeks had a fatty acid pattern similar to those fed 11% fat since weaning.

Comments:

1) The total fatty acid content and the fatty acid composition of liver is fairly stable and does not readily reflect large changes in dietary fat.

2) The fatty acid composition of carcass and abdominal fat changed readily with changes in level of dietary fat. This was attributed to the fatty acid composition of the fat that was fed at 3 different levels, rather than to changes in endogenous synthesis since the dietary fat consisted of 49% C12:0, 19% C14:0, and 9% each of C16:0 and C18:0.

3) Since the rats fed 3/4 as many calories as the control rats had the same fatty acid composition of the fat and liver even though they had lost considerable weight, it appears that certain fatty acids of body fat are not used in preference to others for energy. On the other hand, an excess storage of C12:0 and C14:0 fatty acids did not impair the utilization of others.

4) The percentage of solids in abdominal fat and of total fatty acid in carcass may have been slightly lower in rats fed 3/4 of calories as control animals.

EXPERIMENT II

Procedure:

Weanling 21-day-old male rats of the Long Evans strain were housed individually and were fed, ad libitum, Purina rat chow for 7 weeks. They were then fed purified diets containing 0% (11 rats) or 45% (10 rats) of calories as fat for 5.5 additional weeks. The composition of the diets was the same and the measurements of body weight and food intake were performed as in Experiment I. At 12 weeks post-weaning, the two diet groups were divided so that a) 3 rats were fed weighed rations of the same diet at the level of 17.5 calories/100 gm body weight/day, b) 3 or 4 rats were fed the same diet but restricted to only 14.5 calories/100 gm weight/day, and c) 4 rats were changed to the other diet and fed 14.5 calories/100 gm weight/day for 5.5 weeks more. The day before sacrifice the food cups were withheld from the cages until about 4:30 PM at which time the food was supplied. Sacrifice was by guillotine in the morning. Age at time of sacrifice was 138-145 days. The rats were bled and the liver and abdominal fat were taken as in Experiment I. In addition, all of the epididymal fat was removed for separate analyses. Mesenteric fat and carcass were each weighed, but not analyzed. Chemical analysis for fatty acid composition of liver, abdominal fat and epididymal fat were carried out by the method described in Experiment I. In addition, the activities of seven soluble enzymes were assayed in the 100,000 x g supernatant fractions prepared from homogenates of liver and epididymal fat taken from 3 rats fed the 0% fat diet and 3 rats fed the 45% fat diet (2).

Results:

The results obtained in this experiment with male rats as subjects confirm the previous findings made with female rats. Caloric efficiency was the same whether the 0% or 45% fat diet was consumed--the weight gains were the same with either diet when 17.5 cal/100 gm body weight/day were fed and, likewise,

the weight losses were the same when either diet was fed at 14.5 cal/100 gm body weight/day (Table 8).

The solids content of the liver, abdominal and epididymal fat did not change with the amount of fat in the diet or whether 17.5 or 14.5 cal/100 gm body weight/day were consumed. The total fatty acid content of liver and epididymal fat was not affected by the amount of fat in the diet or the level of caloric intake. It appeared to be a little higher in abdominal fat when the 45% fat diet was consumed (Tables 9 and 10).

The fatty acid composition of the three tissues was the same whether a full ration was fed or one restricted to 80% of the calories. The abdominal and epididymal fat of the rats fed only the 0% fat diet after the period on chow had the same fatty acid composition, however the C12:0 fatty acid was lower and the C18:2 fatty acid was higher in the epididymal fat than in the abdominal fat of rats fed the 45% fat diet at cessation of the chow diet (Table 9).

The consumption of the 45% fat diet rather than the 0% fat diet resulted in large increases in the C12:0 and C14:0 fatty acids in the abdominal and epididymal fat, with the greater differences being seen in the abdominal fat. Changing from a diet containing 0% fat to one containing 45% fat or vice versa resulted in the fatty acid composition of the epididymal and abdominal fat being intermediate to that when 0% or 45% fat diets only were fed.

The effects of the amount of fat in the diet were also seen in the fatty acid composition of the liver but to a less-marked degree (Table 10).

The results of the enzyme assays on liver and epididymal adipose tissue of six male rats fed the 0% or 45% fat diets are shown in Table 11. In liver, all enzymes assayed except hexokinase had lower specific activities when the

45% fat diet was consumed than when the diet was 0% fat. In the adipose tissue, glucose-6-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, citrate cleavage and malic enzymes had lower specific activities when the 45% fat diet was consumed, but pyruvate kinase had a slightly higher specific activity. This latter observation in adipose tissue was contrary to what was found in liver and should be studied at a later date to see if it is significant.

Comments:

It is apparent that the endogenous synthesis of C12:0, 14:0, 14:1, 18:0, and 20:4 fatty acids is low in adipose tissue of abdominal and epididymal fat pads. However, C12:0 and 14:0 fatty acids are readily stored in these tissues when they are provided in the diet, concomitantly, the proportions of C16:0, 16:1, and 18:1 fatty acids are decreased. The endogenous synthesis of C12:0, 14:0, 14:1 and 24:0 fatty acids is also very low in liver, but dietary fat has only a slight effect. This may be attributed to the low level of fatty acids (4-5% wet weight) in liver of which less than one-half is triglyceride. In adipose tissue, on the other hand, fatty acids composed about 86% of the wet weight, mostly as triglycerides (5), the chief storage form of energy.

In adipose tissues the male rats had higher levels of 18:2 and lower 18:1 fatty acids than did the female rats. This is probably the result of the consumption of rat chow by the males for 50 days post weaning while the females consumed only the purified diets since the time of weaning. Hence, the greater storage of C18:2 initially by the male rats in Experiment II.

Since the enzyme data looked interesting but were limited in amount, it was decided to perform another series of enzyme studies on liver and adipose tissue of rats fed diets containing 0% or 45% of the calories as fat. Such an investigation was performed and is given as Experiment III.

EXPERIMENT III

Procedure:

In this study fourteen female and seven male rats of the Long-Evans strain were weaned at 21 days of age and placed in individual cages and fed a chow diet for two months. They were then given diets containing 0% or 45% of the calories as fat for 17-24 days so as to establish a value for food intake per 100 gm body weight per day. The two diet groups were then divided into two sub-groups: a) full-ration (25 cal/100 gm. body weight/day), and b) one-half ration (14 cal/100 gm body weight/day) diets which were fed for 32 days as shown below.

Initial Diet	No. of Rats	Number of rats on final diets			
		Full ration		One-half ration	
		0%	45%	0%	45%
0% fat	8	2	3	-	3
45% fat	8	3	2	3	-
Chow	5*	-	3	-	-

*two rats were kept on chow

The carbohydrate constituent of the diets was modified from one which had only Dextri-Maltose to one which had a 3:1 mixture of cornstarch and Dextri-Maltose. During the final diet period, each rat was given its ration on Monday, Wednesday, and Friday and was weighed each Tuesday and Friday. Safflower oil was given as before. On the day before sacrifice, the final one-day ration was given late in the afternoon.

At sacrifice, liver and fat depots were taken as before and assayed for: a) total solids content, b) total fatty acid content and composition and c) enzyme activities in homogenates. Slices of liver and fat were also prepared and a study of their metabolic throughput was investigated with the use of acetate-1-^{14}C, pyruvate-2-^{14}C and leucine-U-^{14}C. (4).

Results:

The caloric intake and body weight changes in the rats during the final diet period is shown in Table 12. Again the caloric efficiency data are similar for the 0% and 45% fat diets. Body weight losses were the same for males and females but the weight gains appeared to be higher in the males than in the females for the same caloric intake.

Enzyme activities in homogenates prepared from liver and abdominal fat are given in Table 13. These diets appear to have no significant effect on the enzyme activities in abdominal fat tissue, whereas, in liver all of the enzymes tested except hexokinase and α-glycerophosphate dehydrogenase had higher activities when the rats consumed the fat-free diet. These results agree with those obtained in Experiment II regarding the effects of diet on liver enzymes, however, the activities of enzymes in abdominal adipose tissue do not parallel those in epididymal adipose tissue of Experiment II. This could be a reflection of the differences in metabolism between abdominal and epididymal adipose tissue or it could be the result of the differences in the time of feeding in the two experiments. In Experiment II food was removed at 5PM so that these animals were subjected to an overnight fast, whereas in Experiment III food was provided at 5PM and was available to the rats until sacrifice. For conclusive results, another study would have to be undertaken.

The incorporation of the ^{14}C of acetate-1-^{14}C, pyruvate-2-^{14}C, and leucine-U-^{14}C into fatty acids was measured in liver slices and abdominal fat (Table 14). Whereas liver slices prepared from rats fed the fat-free diet incorporated more of the added isotope into fatty acids than did those from the rats fed the 45% fat diet, abdominal fat taken from those two groups of animals did not show large differences*. In liver, the incorporation of the ^{14}C from acetate-1-^{14}C showed

*In the experiments where lipogenesis by adipose tissue was studied from acetate, pyruvate, and leucine, no glucose was added to the incubation medium. In view of the well-known stimulatory effect of glucose on the incorporation of acetate carbon into fatty acid carbon by epididymal adipose tissue (5), it is possible that differences in the rate of lipogenesis between diet groups would have been observed in the presence of glucose. Such experiments are planned.

the highest incorporation into palmitic acid. This fatty acid contained 35-45% of the radioactivity found in the total fatty acids (Table 15). Significant amounts of radioactivity were also recovered in stearic (C18:0) and oleic acids (18:1). The effects of the presence of fat in the diet on the chain lengths of the fatty acids synthesized from acetate were equivocal. The synthesis of palmitic, stearic and oleic acids by liver appears to proceed at a steady rate even though the total incorporation of acetate-1-^{14}C into fatty acids was greater when no fat was administered to the rat.

Comments:

The data concerning the fatty acid composition of the tissues (liver, abdominal, and epididymal fat) in this last experiment is not yet completed. Preliminary evidence, however, confirms previous observations (Experiment I and II) that the fatty acid composition of adipose tissue is readily affected by dietary fat and the fatty acid compositions of liver and abdominal fat are similar in males and females. Epididymal and abdominal fat in the same animal have similar fatty acid compositions.

Thus whereas metabolic activities of liver and adipose tissue are affected by diets high and low in fat, fatty acid composition in the liver does not change as readily as it does in the adipose tissue stores. The composition of fatty acids in adipose tissue reflects more readily the composition of the dietary fat than does that of the liver.

Table 1. DIET COMPOSITION

Component	DIET					
	Percent of Calories			Percent by Weight		
	0% Fat	11% Fat	45% Fat	0% Fat	11% Fat	45% Fat
Casein	23.6	20.4	22.6	22.0	20.0	28.3
Dextri-Maltose[1]	76.5	68.4	32.3	71.8	67.0	40.4
Hydrogenated Fat[2]	0	11.2	45.1	0	5.0	25.1
Salt Mix[3]	0	0	0	4.0	3.5	4.0
Vitamin Mix[4]	0	0	0	0.2	0.2	0.2
Celluflour	0	0	0	2.0	4.3	2.0

[1] Mead Johnson Company: Composition was 42% dextrins, 56% maltose, 2% water.

[2] Plastine, Durkee Famous Foods: Hydrogenated coconut oil. Fatty acid composition: C8:0, 6.1%; C10:0, 5.5%; C12:0, 48.9%; C14:0, 18.7%; C16:0, 9.0%; C18:0, 9.6%; C18:1, 2.1%.

[3] Contained the following in grams: $CaCO_3$, 72.5; $CaHPO_4$, 113.0; Na_2HPO_4, 65.1; KCl, 40.0; $MgSO_4$, 23.0; $MnSO_4 \cdot H_2O$, 1.54; $CuSO_4$, 0.13; ferric citrate, 1.51; $ZnCO_3$, 0.21; and KIO_4, 0.01.

[4] Contained the following in grams: choline bitartrate, 13.500, Vitamin A palmitate (250,000 IU/gm), 0.080; Vitamin D_2 (500,000 IU/gm), 0.030; D-α-tocopherol acid succinate (890 IU/gm), 0.675; menadione sodium bisulfite, 0.002; thiamine hydrochloride 0.0125; riboflavin, 0.025; pyridoxine hydrochloride, 0.012; niacinamide, 0.150; calcium pantothenate, 0.080; Vitamin B_{12} (0.1% in gelatin), 0.005; folic acid, 0.005.

Table 2. BODY WEIGHT CHANGES IN ADULT FEMALE RATS AS THE RESULT OF FEEDING TWO CALORIC LEVELS OF SYNTHETIC DIETS CONTAINING 0%, 11%, or 45% OF CALORIES AS FAT

Diet, % Fat		No. of. Rats	Final Diet Period			
Initial	Final		Time	Initial Body Wt.	Cal. Eaten	Body Wt. Change
			days	grams	per 100 gm body wt/day	gm/day
0	0	1	41	228	23	+ 0.44
45	0	3	41	227	23	+ 0.55
11	11	5	37	209	25	+ 0.43
45	11	5	37	206	25	+ 0.51
0	45	2	40	230	25	+ 0.53
11	45	5	37	200	25	+ 0.22
45	45	5	37	212	25	+ 0.0
0	0	3	41	226	19	- 0.81
45	0	2	42	239	19	- 0.77
11	11	4	38	209	19	- 0.90
0	45	3	41	221	17	- 0.69
45	45	5	39	212	19	- 0.89

Table 3. COMPOSITION OF FATTY ACIDS IN LIVER OF RATS FED SYNTHETIC DIETS PROVIDING 0%, 11%, or 45% OF CALORIES AS FAT

Diet, % Fat		No. of Rats	Solids	Fatty Acids	Fatty Acids[1], % Total Fatty Acids											
Initial	Final				12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4	21:1	21.5	24:0
			% wet wt.	% Solids												
0	0	5	32	12	0.1	0.3	0.1	18.5	4.9	17.4	29.3	3.3	18.2	4.0	1.3	2.6
45	0	5	33	14	0.2	0.8	0.2	19.6	6.7	14.4	32.4	3.5	15.3	3.8	1.1	2.0
11	11	5	30	14	0.3	1.0	0.2	19.0	5.6	16.7	28.9	3.6	16.9	3.5	1.2	2.7
11	11[2]	6	31	14	0.2	0.7	0.2	18.4	4.9	20.0	27.6	4.0	17.0	4.1	1.5	2.0
45	11	5	31	14	0.4	1.1	0.2	18.7	5.7	16.8	29.9	4.1	14.4	5.2	1.6	2.1
0	45	5	32	12	0.8	1.5	0.2	18.9	4.2	18.1	26.2	4.0	17.3	4.5	1.6	2.9
11	45	5	31	12	0.6	1.5	0.2	18.0	3.8	21.2	24.3	4.0	15.7	5.7	1.7	2.8
45	45	5	30	12	0.6	1.4	0.2	17.4	3.5	21.1	22.5	4.2	18.0	5.5	1.9	2.9
45	45[2]	8	31	12	1.0	2.1	0.4	18.8	4.8	17.6	26.8	4.7	15.2	4.7	1.4	2.5

[1] Designated as number of C atoms in chain followed by the number of double bonds.

[2] These rats were fed 75% of the full diet ration for the final diet period.

Table 4. COMPOSITION OF FATTY ACIDS IN ABDOMINAL ADIPOSE TISSUE OF RATS FED SYNTHETIC DIETS CONTAINING 0%, 11%, or 45% OF CALORIES AS FAT

Diet, % Fat		No. of		Fatty	Fatty Acids[1], % Total Fatty Acids								
Initial	Final	Rats	Solids	Acids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4
			% wet wt.	%solids									
0	0	5	93	92	0.1	1.4	0.3	24.5	10.7	3.6	56.6	1.8	0.4
45	0	5	93	93	4.6	5.8	0.9	25.5	11.9	3.5	45.7	1.9	0.2
11	11	5	92	91	4.5	5.6	0.9	26.0	11.5	3.2	43.1	1.8	0.3
11	11[2]	5	88	87	5.2	5.6	0.8	24.6	9.5	4.5	48.0	1.7	0.4
45	11	5	93	92	7.6	7.7	1.0	24.4	10.7	3.5	42.4	1.8	0.4
0	45	5	94	92	10.8	9.4	1.0	23.3	8.2	4.2	41.2	1.5	0.2
11	45	5	93	92	11.9	10.2	0.9	23.3	8.4	4.0	40.4	1.6	0.3
45	45	5	89	92	12.8	12.1	1.0	22.6	7.5	4.4	36.1	1.5	0.3
45	45[2]	7	84	91	14.5	12.5	1.1	21.7	6.5	5.1	36.1	1.7	0.4

[1] Designated as number of C atoms in chain followed by the number of double bonds.

[2] These rats were fed 75% of the full diet ration for the final diet period.

Table 5. COMPOSITION OF FATTY ACIDS IN CARCASS OF RATS FED SYNTHETIC DIETS CONTAINING 0%, 11%, OR 45% CALORIES AS FAT

Diet, % Fat		No.of	Fatty Acids	Fatty Acids[1], % Total Fatty Acids								
Initial	Final	Rats	% Wet Wt.	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4
0	0	5	11	0.1	1.7	0.5	23.3	13.2	3.7	50.3	2.2	2.8
45	0	5	12	3.7	5.0	1.1	24.6	14.2	3.6	42.8	2.3	2.0
11	11	5	11	4.6	5.6	1.0	23.5	12.8	3.6	42.0	2.5	2.4
11	11[2]	4	7	3.2	4.5	0.9	22.4	11.2	4.5	45.2	2.6	3.1
45	11	5	12	5.3	6.7	1.2	23.4	12.2	3.7	41.0	2.2	1.8
0	45	5	10	14.1	8.3	0.9	20.4	9.0	4.1	37.6	2.5	2.5
11	45	5	9	10.1	9.5	1.0	21.1	8.8	4.7	37.5	2.4	2.5
45	45	5	9	12.0	10.9	1.1	20.8	7.9	5.2	34.9	2.4	2.7
45	45[2]	7	8	12.7	10.8	1.2	20.9	8.0	5.4	37.8	2.5	2.8

[1] Designated as number of C atoms in chain followed by the number of double bonds.

[2] These rats were fed 75% of the full diet ration for the final diet period.

Table 6. SUMMARY OF FATTY ACID COMPOSITION OF LIVER ACCORDING TO DIET GROUPS[1]

Diet Groups		% of Wet Wt.	TFA,%	Fatty Acids[2], % Total Fatty Acids											
Initial	Final	Solids	Solids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4	21:1	21.5	24:0
0%	0%	32	12	0.1	0.3	0.1	18.5	4.9	17.4	29.3	3.3	18.2	4.0	1.3	2.6
11% 45%	11% 0%or11%	31	14	0.2	0.9	0.2	19.0	6.1	16.0	29.5	3.7	15.0	3.7	1.7	2.3
45%	45%	30	12	0.6	1.4	0.2	17.4	3.5	21.1	22.5	4.2	18.0	5.5	1.9	2.9
0%or 11%	45%	31	12	0.7	1.5	0.2	18.4	4.0	19.6	25.2	4.0	16.5	5.1	1.7	2.8

[1] Composite values for dietary regimens. [2] Designated as number of C atoms in chain followed by the number of double bonds.

Table 7. SUMMARY OF FATTY ACID COMPOSITION OF ABDOMINAL FAT AND CARCASS LIPIDS ACCORDING TO DIET GROUPS[1]

Diet Groups		Tissue[2]	% of Wet Wt.	TFA	Fatty Acids[3], % Total Fatty Acids								
Initial	Final		Solids	% Solids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4
0%	0%	A	93	92	0.1	1.4	0.3	24.5	10.7	3.6	56.6	1.8	0.4
		C	--	11[4]	0.1	1.7	0.5	23.3	13.2	3.7	50.3	2.2	2.8
11%	11%	A	93	92	5.5	6.3	0.9	25.3	11.5	3.4	43.7	1.8	0.3
45%	0% or 11%	C	--	12[4]	4.5	5.7	1.1	24.0	13.0	3.6	42.0	2.4	3.1
45%	45%	A	89	92	12.8	12.1	1.0	22.6	7.5	4.4	36.1	1.5	0.3
		C	--	9[4]	12.0	10.9	1.1	20.8	7.9	5.2	34.9	2.4	2.7
0% or 11%	45%	A	93	92	11.3	9.8	0.9	23.3	8.3	4.1	40.8	1.6	0.3
		C	--	9[4]	12.1	8.9	1.0	20.7	8.9	4.4	37.6	2.4	2.5

[1] Composite values for dietary regimens.
[2] A-Abdominal fat; C-Carcass lipids.
[3] Designated as number of C atoms in chain followed by the number of double bonds.
[4] % wet weight

Table 8. BODY WEIGHT CHANGES IN ADULT MALE RATS AS THE RESULT OF FEEDING TWO CALORIC LEVELS OF SYNTHETIC DIETS PROVIDING 0% OR 45% OF CALORIES AS FAT

Diet % Fat		No. of	Final Diet Period			
Initial	Final	Rats	Time	Initial Body Wt.	Calories Eaten	Body Wt. Change
			days	grams	per 100 gm body wt/day	gm/day
0	0	3	38	404	17.6	+ 0.39[1]
45	45	3	37	421	17.5	+ 0.99[2]
0	0	4	35	375	14.6	- 1.10
45	0	4	36	423	14.4	- 1.39
45	45	3	36	423	14.5	- 1.42
0	45	4	35	415	14.3	- 1.20

[1]average of 0.0, 0.53 and 0.63

[2]average of 0.59, 0.95 and 1.43

Table 9. COMPOSITION OF FATTY ACIDS IN ABDOMINAL AND EPIDIDYMAL ADIPOSE OF MALE RATS FED DIETS CONTAINING 0% OR 45% OF CALORIES AS FAT

Diet		No. of	Solids,	TFA,	Fatty Acids, % Total Fatty Acids								
Initial	Final	rats	% Wet Wt.	% Solids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4
ABDOMINAL ADIPOSE TISSUE													
45	45[a]	3	96	94	17.5	13.2	0.7	23.6	5.6	4.9	28.1	5.9	0.3
45	45[b]	3	94	92	18.8	14.0	0.7	21.8	4.3	5.9	28.1	5.9	0.3
0	45[b]	4	95	93	10.6	8.6	0.6	20.1	7.3	4.7	38.0	6.3	0.3
45	0[b]	4	94	91	6.9	6.9	0.6	26.2	8.9	4.3	39.4	6.3	0.3
0	0[a]	3	95	90	0.5	2.5	0.4	30.7	10.5	3.1	43.1	6.2	0.3
0	0[b]	4	92	90	0.7	2.2	0.4	28.8	11.1	3.6	45.3	7.3	0.3
EPIDIDYMAL ADIPOSE TISSUE													
45	45[a]	3	94	94	13.4	11.7	0.9	22.5	7.2	4.1	31.0	8.9	0.3
45	45[b]	3	92	92	14.9	12.3	1.0	19.8	6.3	4.3	30.8	10.3	0.2
0	45[b]	4	94	92	7.0	6.3	0.7	23.0	11.5	3.1	36.7	10.7	0.3
45	0[b]	4	94	94	9.4	7.9	1.0	22.7	9.8	3.5	35.5	9.6	0.3
0	0[a]	3	94	92	0.5	3.1	0.5	27.8	15.4	2.6	41.6	7.0	0.1
0	0[b]	4	91	90	0.8	2.8	0.5	27.3	12.4	3.2	42.4	9.8	0.2

[a] Received 17.5 cal/100 gm body wt/day

[b] Received 14.5 cal/100 gm body wt/day

Table 10. COMPOSITION OF FATTY ACIDS IN LIVER OF MALE RATS FED DIETS CONTAINING 0% OR 45% OF CALORIES AS FAT

Diet		No. of rats	Solids, % Wet Wt.	TFA % Solids	Fatty Acids, % Total Fatty Acids											
Initial	Final				12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4	21:1	21.5	24:0
45	45[a]	3	33	16	1.0	3.8	0.3	23.8	4.0	16.8	25.0	5.0	15.8	1.8	1.8	1.2
45	45[b]	3	31	13	0.7	2.4	0.2	20.7	3.5	18.2	23.2	7.0	19.6	2.1	1.7	1.3
0	45[b]	4	32	15	1.1	3.2	0.2	24.8	4.8	12.2	27.2	6.0	12.5	1.3	1.1	0.5
45	0[b]	4	32	13	0.1	0.8	0.1	21.8	6.9	11.9	31.4	4.7	18.3	1.7	1.2	1.3
0	0[a]	3	32	12	0.1	0.8	0.1	27.0	9.2	11.9	29.4	3.1	13.9	2.8	1.2	0.7
0	0[b]	4	32	11	0.1	0.5	0.1	22.3	6.1	13.0	28.7	6.4	19.8	1.5	1.2	0.8

[a] Received 17.5 cal/100 gm body wt/day

[b] Received 14.5 cal/100 gm body wt/day

Table 11. ENZYME ACTIVITY[1] IN LIVER AND EPIDIDYMAL ADIPOSE TISSUE OF SIX MALE RATS FED PURIFIED DIETS CONTAINING 0% OR 45% OF CALORIES AS FAT

Enzyme	Liver		Epididymal Adipose Tissue	
	Diet, % Fat		Diet, % Fat	
	0% (3)[2]	45% (3)	0% (3)	45% (3)
Hexokinase	5	4	4	5
Glucokinase	67	31	---	---
Glucose-6-phosphate dehydrogenase	105	27	62	25
6-Phosphogluconate dehydrogenase	72	45	23	18
Pyruvate kinase	430	140	54	64
α-Glycerolphosphate dehydrogenase	642	553	484	226
Citrate cleavage enzyme	36	1	7	3
Malic enzyme	88	19	179	10

[1]All results are presented as mμmoles of pyridine nucleotide oxidized or reduced per mg 100,000 x g supernatant protein per minute (specific activity).

[2]Number in parenthesis represents the number of rats in each group.

Table 12.BODY WEIGHT CHANGES IN YOUNG RATS AS THE RESULT OF FEEDING TWO CALORIC LEVELS OF DIETS PROVIDING 0% OR 45% OF CALORIES AS FAT

Diet, % Fat		No. of	Time	Final Diet Period					
				Initial wt, gms		Cal/100 gm body wt/day		Weight Change	
Initial	Final	Rats	Days	Male	Female	Male	Female	Male	Female
								gm per day	
Chow	Chow	3	31	408	290	----	----	+ 1.0	+ 0.25
0	0	2	32	363	265	27.0	21.8	+ 0.44	+ 0.25
45	0	3	32	354	240	28.0	23.2	+ 0.81	+ 0.24
0	45	3	32	375	232	21.0	24.0	+ 0.78	+ 0.25
Chow	45	3	31	300	231	24.7	24.8	+ 1.85	+ 0.26
45	45	2	32	---	230	----	23.4	------	- 0.12
0	45	3	32	384	231	13.0	14.0	- 1.60	- 1.93
45	0	3	32	453	237	14.5	14.6	- 1.97	- 1.85

Table 13. ACTIVITIES[1] OF SOLUBLE ENZYMES IN LIVER AND ABDOMINAL FAT HOMOGENATES OF RATS FED DIETS PROVIDING 0%, 6%, OR 45% OF CALORIES AS FAT

Final Diet	0% Fat		Chow	45% Fat		
Initial Diet	0%	45%	Chow	Chow	0%	45%
No. of Rats	2	3	2	3	3	2
Liver						
Hexokinase	7	6	5	5	5	5
Glucokinase	67	75	27	31	25	26
Glucose-6-phosphate dehydrogenase	183	99	30	91	60	77
6-Phosphogluconate dehydrogenase	111	124	28	43	58	62
Pyruvate kinase	1252	948	301	507	537	427
α-Glycerolphosphate dehydrogenase	596	536	367	545	487	453
Citrate cleavage	42	49	8	19	19	21
Malic enzyme	68	77	14	55	46	36
Abdominal Fat						
Hexokinase	35	27	23	27	16	17
Glucokinase	---	---	---	---	---	---
Glucose-6-phosphate dehydrogenase	157	100	179	124	137	70
6-Phosphogluconate dehydrogenase	87	37	28	59	51	31
Pyruvate kinase	347	259	244	420	297	367
α-Glycerolphosphate dehydrogenase	162	95	666	196	173	254
Citrate cleavage	3	19	13	10	0	5
Malic enzyme	534	152	86	267	148	104

[1]All results are presented as mμmoles of pyridine nucleotide oxidized or reduced per mg 100,000 x g supernatant protein per minute (specific activity).

Table 14. INCORPORATION OF THE ^{14}C OF ACETATE-1-^{14}C, PYRUVATE-2-^{14}C, AND LEUCINE-U-^{14}C INTO FATTY ACIDS BY SLICES OF LIVER AND ABDOMINAL ADIPOSE TISSUE OF RATS FED DIETS CONTAINING DIFFERENT AMOUNTS OF FAT.

Substrate	Diet, % Fat		% of added isotope recovered in Fatty Acids from slices of:	
	Initial	Final (4-5 weeks)	Liver[1]	Abdominal Adipose Tissue[2]
Acetate-1-^{14}C	45	45	1.23 (2)	0.24 (2)
	0	45	0.72 (3)	0.28 (3)
	Chow	45	1.41 (3)	----
	0	0	5.45 (2)	0.55 (2)
	45	0	5.75 (3)	0.43 (2)
Pyruvate-2-^{14}C	45	45	0.46 (2)	0.21 (2)
	0	45	0.18 (2)	0.32 (3)
	Chow	45	0.53 (3)	0.24 (3)
	0	0	1.79 (2)	0.34 (2)
	45	0	2.67 (3)	0.56 (2)
Leucine-U-^{14}C	45	45	0.37 (2)	0.25 (2)
	0	45	0.17 (3)	0.36 (1)
	Chow	45	0.30 (3)	0.30 (3)
	0	0	0.57 (2)	----
	45	0	0.93 (3)	0.32 (3)

[1]In the experiments with liver slices, 100 mg of slices were incubated with 1 ml of Krebs-Henseleit bicarbonate buffer at pH 7.3 (3) containing either 2 μmoles of Acetate-1-^{14}C, 5 μmoles of Pyruvate-2-^{14}C or 1 μmole of L-Leucine, uniformly labeled with ^{14}C for 3 hrs at 37° with a mixture of 95% O_2 and 5% CO_2 as gas phase. The isolation of the fatty acids and assay for ^{14}C activity has been described elsewhere (4). All values are given as the averages (per cent of added isotope recovered in fatty acid fraction) of separate determinations with individual rats. The number of rats used is given in parenthesis.

[2]In the experiments with adipose tissue, 200 mg of slices were incubated with 1 ml of the same buffer containing either 2 μmoles of Acetate-1-^{14}C, 5 μmoles of Pyruvate-2-^{14}C or 1 μmole of L-Leucine, uniformly labeled with ^{14}C for 3 hrs. at 37°. A mixture of 95% O_2 and 5% CO_2 served as gas phase. Subsequent analysis given above.

Table 15. INCORPORATION OF ACETATE-1-^{14}C INTO FATTY ACIDS BY LIVER SLICES OF RATS MAINTAINED ON SYNTHETIC DIETS PROVIDING 0% OR 45% OF THE CALORIES AS FAT

Diet, % Fat	45% only		0% only		45% then 0%	
rat no.	25	34	40	32	38	27
% incorp.	1.32	1.14	7 91	2.98	12.36	3.34
Fatty Acid:	% of cpm in fatty acid					
14:0	3	6	7	9	4	5
16:0	53	24	43	44	46	35
16:1	0	0	3	7	10	4
18:0	18	31	29	6	15	35
18:1	12	8	13	29	21	16
18:2	2	2	1	<1	<1	0
20:4	3	15	<1	0	0	0

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